Despite Predominance of Uropathogenic/Extraintestinal Pathotypes Among Travel-acquired Extended-spectrum -Lactamase–producing Escherichia coli, the Most Commonly Associated Clinical Manifestation Is Travelers' Diarrhea

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Background. One-third of the 100 million travelers to the tropics annually acquire extended-spectrum β-lactamase (ESBL)–producing Enterobacteriaceae (ESBL-PE), with undefined clinical consequences.

Methods. Symptoms suggesting Enterobacteriaceae infections were recorded prospectively among 430 Finnish travelers, 90 (21%) of whom acquired ESBL-PE abroad. ESBL-PE isolates underwent polymerase chain reaction–based detection of diarrheagenic *Escherichia coli* (DEC) pathotypes (enteroaggregative *E. coli* [EAEC], enteropathogenic *E. coli* [EPEC], enterotoxigenic *E. coli* [ETEC], enteroinvasive *E. coli*, and Shiga toxin–producing *E. coli*), and extraintestinal pathogenic/uropathogenic *E. coli* (ExPEC/UPEC). Laboratory-confirmed ESBL-PE infections were surveyed 5 years before and after travel.

Results. Among the 90 ESBL-PE carriers, manifestations of Enterobacteriaceae infection included travelers' diarrhea (TD) (75/90 subjects) and urinary tract infection (UTI) (3/90). The carriers had 96 ESBL-producing *E. coli* isolates, 51% exhibiting a molecular pathotype: 13 (14%) were DEC (10 EAEC, 2 EPEC, 1 ETEC) (12 associated with TD) and 39 (41%) ExPEC/UPEC (none associated with UTI). Of ESBL-PE, 3 (3%) were ExPEC/UPEC-EAEC hybrids (2 associated with diarrhea, none with UTI). Potential ESBL-PE infections were detected in 15 of 90 subjects (17%). The 10-year medical record survey identified 4 laboratory-confirmed ESBL-PE infections among the 430 travelers, all in subjects who screened ESBL-PE negative after returning home from their index journeys but had traveled abroad before their infection episodes.

Conclusions. Half of all travel-acquired ESBL-producing *E. coli* strains qualified molecularly as pathogens. Extraintestinal and uropathogenic pathotypes outnumbered enteric pathotypes (41% vs 14%), yet the latter correlated more closely with symptomatic infection (0% vs 92%). Despite more ESBL-PE strains qualifying as ExPEC/UPEC than DEC, travel-acquired ESBL-PE are more often associated with TD than UTI.

Keywords. extended-spectrum beta-lactamase; ESBL; travel; DEC; ExPEC.

International travel drives the global spread of antimicrobial-resistant organisms from high- to low-risk regions [1]. Visitors to the (sub)tropics have been well documented to acquire multidrug-resistant (MDR) intestinal bacteria: 20%–70% carry extended-spectrum β-lactamase (ESBL)–producing Enterobacteriaceae (ESBL-PE) at return [2–10].

Colonizing intestinal MDR bacteria may have considerable implications for both the community and colonized individuals. Such strains can spread silently to close contacts within a community, in healthcare settings, and eventually even worldwide [11]. Colonized travelers usually remain asymptomatic [1], but can develop severe, even life-threatening MDR infections [12, 13]. Infections with MDR strains result in greater morbidity, mortality, and economic losses than those caused by susceptible strains [12]. Although one report suggested that imported ESBL-PE strains have low pathogenicity [14], no prospective study has assessed the clinical infection risk such strains pose.

In studies exploring clinical ESBL-PE isolates, urinary tract infection (UTI) has appeared to be the most common type of infection caused by travel-acquired MDR bacteria [15]. In a recent study, 9% of travel-acquired ESBL-PE strains carried UTI-associated virulence factors; data on possible related symptoms

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of cystitis were not reported [14]. Additionally, retrospective studies have identified international travel as a risk factor for UTI caused by ESBL-PE [15, 16].

The most common health problem travelers encounter is travelers' diarrhea (TD) [17]. In numerous recent studies utilizing multiplex quantitative polymerase chain reaction (qPCR) assays, the various diarrheagenic *Escherichia coli* (DEC) pathotypes have been the most frequently detected stool pathogens in TD; multiple pathogens often co-occur [18, 19]. Although DEC and other enteric pathogens are also found among asymptomatic travelers, the prevalence is higher among those with TD [18, 19]. Many studies have explored acquisition of ESBL-PE during travel [1–10, 20], and others have examined TD pathogens [18, 19] and their resistance profiles [21, 22]. However, none have investigated whether some travel-acquired ESBL-PE isolates actually are TD pathogens, as might be expected, given the rising prevalence of antibiotic resistance, including ESBL production, among TD pathogens [23].

Here, we studied the clinical significance of travel-acquired ESBL-PE. Specifically, we prospectively surveyed Finnish international travelers for symptoms suggesting Enterobacteriaceae infections (eg, UTI and TD), screened their feces for travel-acquired ESBL-PE, determined whether the colonizing ESBL-PE strains qualified molecularly as DEC or extraintestinal pathogenic/uropathogenic *E. coli* (ExPEC/UPEC), and compared clinical data with molecular data. We also reviewed subjects' medical records for clinical ESBL-PE infections during the 5 years before and after the index travel episode.

MATERIALS AND METHODS

Study Design, Volunteers, Samples, and Travel Destinations

As reported elsewhere, 430 incipient travelers were recruited prospectively at the Travel Clinic of Aava Medical Centre [7] prior to visiting the tropics. Each subject provided a pre- and posttravel stool sample to be screened for ESBL-PE, completed a questionnaire before and after their journey, and kept a diary while abroad. The Helsinki University Hospital ethics committee approved the protocol. All subjects provided written informed consent.

Subjects whose posttravel but not pretravel stools yielded ESBL-PE were considered to have travel-acquired ESBL-PE. Subjects with vs without travel-acquired ESBL-PE constituted the $ESBL^+$ and $ESBL^-$ groups, respectively. We have previously reported risk factors for acquiring ESBL-PE [7]; all subjects but 1 were the same in both studies.

To identify all possible symptomatic ESBL-PE infections experienced during travel, we searched for symptoms suggesting Enterobacteriaceae infections (described below), then investigated whether subjects with such symptoms had travel-acquired ESBL-PE, and whether the strains carried pathotype-defining markers for DEC, ExPEC, or UPEC. A theoretical maximum rate of infections caused by travel-acquired ESBL-PE was calculated. Case criteria were as follows: (1) symptoms of an Enterobacteriaceae infection; (2) acquisition of ESBL-PE during travel; and (3) for TD, the travel-acquired ESBL-PE strain qualified as DEC. For UTI and other Enterobacteriaceae infections, no pathotype restrictions applied, since non-ExPEC/UPEC also can cause UTI. Despite uncertain causality, this approach should identify the maximum rate of infections caused by travel-acquired ESBL-PE (Figure 1).

In addition to exploring ESBL-PE infections during travel, we sought for relevant results in the subjects' medical records. To assess whether the index travel increased the risk of clinical ESBL-PE infections, the search included 5 years before index travel and 5 years after it.

Symptoms of Potential Enterobacteriaceae Infections

As noted above, the posttravel questionnaire and diaries captured whether while abroad the subject had experienced symptoms suggesting Enterobacteriaceae infection (eg, TD or UTI). TD was defined as passage of ≥ 3 loose or liquid stools per day, or more frequently than normal for the individual [24]. Cystitis was defined as dysuria and/or frequency and/or urgency [25], and pyelonephritis as a febrile illness (with or without urinary symptoms) without diarrhea or respiratory symptoms or other obvious cause of fever.

Molecular Identification of Diarrheal and Extraintestinal Pathogens

As detailed previously, fecal ESBL-PE were isolated and characterized using established methods with culture on chromID ESBL (bioMérieux, Marcy-l'étoile, France), followed by double-disk synergy (Oxoid, Thermo Fisher Scientific, Hampshire, United Kingdom) test for cefotaxime (30 μg), ceftazidime (30 μg), and cefpodoxime (30 μg), alone or with clavulanic acid (10 μg), and species identification by Vitek GN (bioMérieux) [7]. Susceptibility testing for ciprofloxacin, cotrimoxazole, nitrofurantoin, tobramycin, ertapenem, imipenem, and meropenem was performed with Etest (bioMérieux) according to criteria set by the European Committee on Antimicrobial Susceptibility Testing 5.0 (2018; www.eucast.org). β-Lactamase genes (TEM, OXA, SHV, CTX-M) and plasmid-mediated AmpC β-lactamase genes (DHA, CIT) were identified with multiplex PCR [26].

To identify DEC, isolates underwent multiplex qPCR screening for 5 DEC pathotypes—enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and Shiga toxin–producing *E. coli* (STEC)—by a method described previously in detail [27]. To identify ExPEC and UPEC, isolates underwent multiplex PCR screening for pathotype-defining extraintestinal virulence genes [28]. Isolates were designated as ExPEC if positive for ≥2 of *papAH* and/or *papC* (P fimbriae), *sfa/foc* (S and

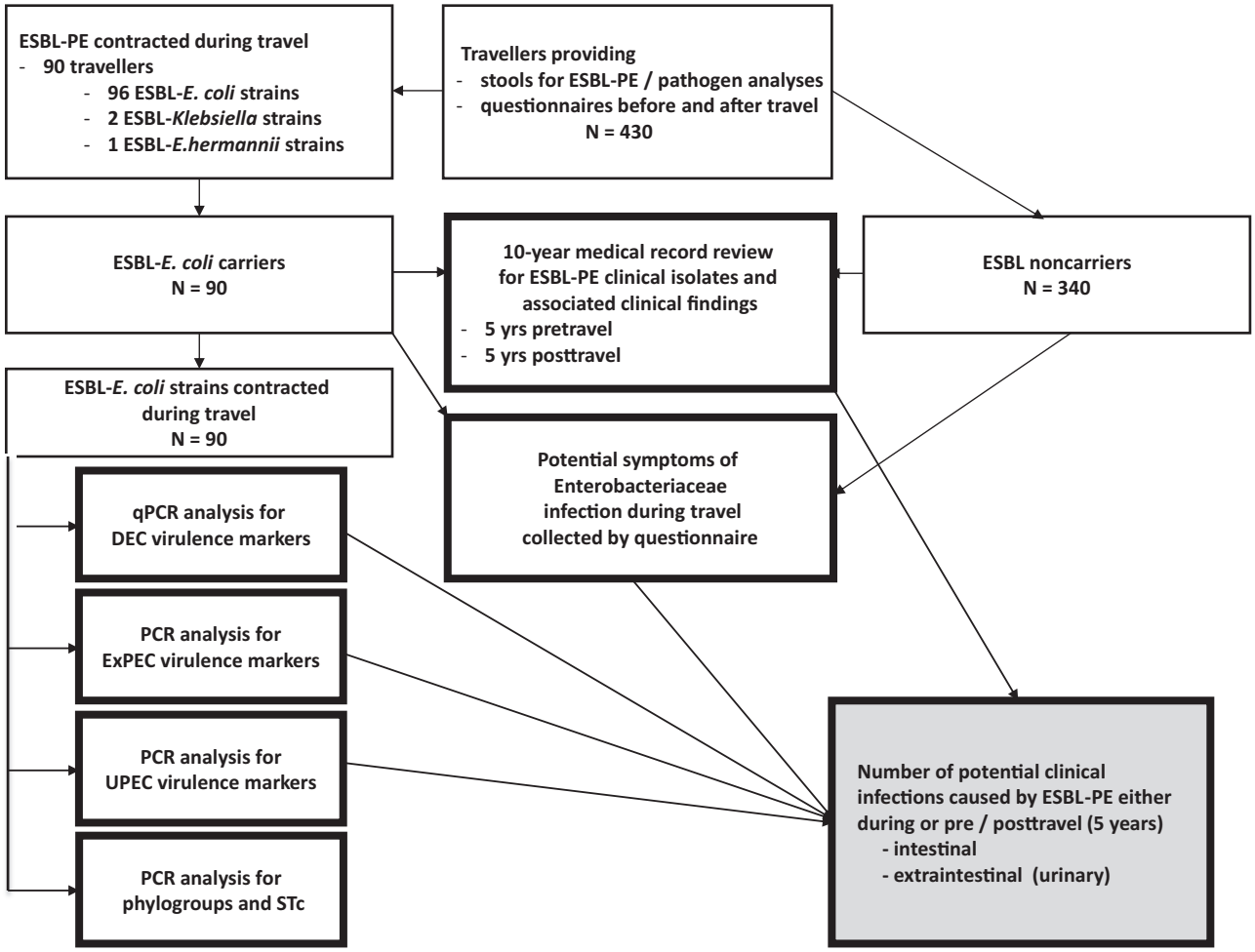

Figure 1. Study protocol flowchart. Shown are the 2 groups of volunteers with respect to acquisition of extended-spectrum β-lactamase–producing Enterobacteriaceae (ESBL-PE) during travel. For each group, subjects were surveyed regarding symptoms typical of clinical Enterobacteriaceae infections, and travel-acquired ESBL-PE isolates were tested for pathotype-defining virulence markers for diarrheagenic *Escherichia coli*, uropathogenic *E. coli*, and extraintestinal pathogenic *E. coli*. Additionally, a medical record survey (spanning 10 years: 5 years before and 5 years after travel) was conducted to screen for laboratory-confirmed extraintestinal infections caused by ESBL-PE. Abbreviations: DEC, diarrheagenic *Escherichia coli*; *E. coli*, *Escherichia coli*; *E. hermannii*, *Escherichia hermannii*; ESBL, extended-spectrum β-lactamase; ESBL-PE, extended-spectrum β-lactamase–producing Enterobacteriaceae; ExPEC, extraintestinal pathogenic *Escherichia coli*; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; STc, sequence type complex; UPEC, uropathogenic *Escherichia coli*.

F1C fimbriae), *afa/dra* (Dr-binding adhesins), *iutA* (aerobactin system), and *kpsM* II (group 2 capsule), and as UPEC if positive for ≥2 of *vat* (vacuolating toxin), *chuA* (heme uptake), *fyuA* (yersiniabactin system), and *yfcV* (adhesin). Isolates could exhibit multiple pathotypes (Table 1).

Major *E. coli* phylogenetic group was defined by multiplex PCR [29], and sequence type complex (STc; group of closely related sequence types [STs]) by a combination of STc-specific PCR assays and *fumC*/*fimH* typing [30].

Additional Medical Record Screening for Severe Extraintestinal ESBL-PE Infections

To identify laboratory-confirmed ESBL-PE infections for 5 years both before and after travel, we surveyed the subjects' laboratory records for ESBL-PE clinical isolates between 1 March 2004 and 30 February 2015, and interviewed each patient with positive findings for additional travel history. The Helsinki University Hospital medical records include results from the Helsinki University Hospital Laboratory (HUSLAB), which serves all public healthcare institutions in the Uusimaa region (1.6 million inhabitants). In Finland, nearly all patients who are severely ill or need blood cultures are treated in public hospitals. Since 2010, patients hospitalized outside the Nordic countries within the prior year have been screened for MDR organisms upon hospital admission.

Statistical Methods

Statistical analyses were performed with SPSS software version 22.0 (SPSS Inc, Armonk, New York). The χ^2 test or Fisher exact test was used to compare categorical variables.

Table 1. Pathotypes of 96 Travel-acquired Extended-spectrum β-Lactamase–producing *Escherichia coli* Strains

Category and Pathotype(s)	No. (% of 96)
DEC[a] only (ie, not ExPEC/UPEC)	
Any	10 (10)
EAEC	7 (7)
EPEC	2 (2)
ETEC	1 (1)
ExPEC/UPEC only (ie, not DEC)	
Any	36 (38)
ExPEC, +/– UPEC	22 (23)
UPEC, +/– ExPEC	34 (35)
ExPEC, not UPEC	2 (2)
UPEC, not ExPEC	14 (14)
ExPEC + UPEC	20 (21)
DEC + ExPEC/UPEC	
Any	3 (3)
EAEC + UPEC	2 (2)
EAEC + ExPEC + UPEC	1 (1)
Other pathotype combinations	
DEC or UPEC or ExPEC	49 (51)
DEC, +/– UPEC/ExPEC	13 (13)
ExPEC/UPEC, +/– DEC	39 (40)
ExPEC, +/– DEC	23 (24)
UPEC, +/– DEC	37 (38)
No pathotype detected	47 (49)

Abbreviations: +/–, with/without; DEC, diarrheagenic *Escherichia coli*; EAEC, enteroaggregative *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; ExPEC, extraintestinal pathogenic *Escherichia coli*; UPEC, uropathogenic *Escherichia coli*.

[a]Five DEC pathotypes (enteroaggregative, enteropathogenic, enterotoxigenic, enteroinvasive, and Shiga toxin–producing *E. coli*).

RESULTS

Description of Travelers, Travels, and Travel-acquired ESBL-PE

The demographics, destinations, and ESBL-PE colonization status of the 430 prospectively recruited international travelers have been reported previously [7]. The 90 (21%) with travel-acquired ESBL-PE constituted the ESBL$^+$ group. Of these, 61% were females and 66% were aged <50 years; the median age was 36.5 years (interquartile range [IQR], 28–58 years), and the median journey duration was 16.5 days (IQR, 12–27 days) (Table 2). The 90 posttravel stools yielded 99 travel-acquired ESBL-PE strains, of which 96 were *E. coli*, 2 *Klebsiella oxytoca*, and 1 *Escherichia hermannii*. The 3 non–*E. coli* isolates were from separate subjects, each also with ESBL-producing *E. coli*. The remaining 340 (79%) subjects constituted the ESBL$^-$ group.

Symptoms of Enterobacteriaceae Infection

The only symptoms suggesting Enterobacteriaceae infection reported by ESBL$^+$ group members were those of TD and UTI. Of these subjects, 75 of 90 (83%) had TD and 3 of 90 (3%) had UTI (specifically, cystitis), the latter including 2 of 49 (4%) females. For comparison, in the ESBL$^-$ group, 213 of 340 (63%) subjects had TD and 13 of 340 (4%) had UTI.

Diarrheal Pathotypes

Of the 96 ESBL-producing *E. coli* isolates, 13 (14%) qualified as DEC, including 10 EAEC, 2 EPEC, and 1 ETEC (no STEC or EIEC), each from a different subject (Table 1). Of the 13 subjects with ESBL-producing DEC, 12 (92%) had experienced TD while abroad. Eight (62%) had visited South Asia, for which the DEC fraction among ESBL-PE isolates was 8 of 29 (28%), vs 5 of 67 (7%) for all other regions combined ($P = .02$) (Table 2).

Extraintestinal and Combined Pathotypes

Of the 96 ESBL-producing *E. coli* fecal isolates, 39 (41%) qualified as ExPEC and/or UPEC, that is, presumptive extraintestinal pathogens (Table 1). Of these, 3 (8%) carried markers of combined intestinal and extraintestinal pathotypes (2 EAEC + ExPEC, 1 EAEC + ExPEC + UPEC).

Antibiotic Resistance Profiles

Among the 96 isolates, resistance was common to tobramycin, ciprofloxacin, and cotrimoxazole, but rare or absent to carbapenems and nitrofurantoin (Table 3). Resistance prevalence, which varied significantly by pathotype for tobramycin, ciprofloxacin, and cotrimoxazole, was lowest among DEC isolates, intermediate among pathotype-negative isolates, and highest among ExPEC/UPEC-only isolates ($P < .01$ for all comparisons of DEC isolates vs ExPEC/UPEC-only isolates).

Phylogroups

Phylogroup distribution and extraintestinal virulence gene content varied significantly by pathotype (Table 4). Most non-ExPEC/UPEC isolates belonged to phylogroups A and B1, whereas nearly all ExPEC/UPEC isolates belonged to groups D and F. Likewise, STc10 predominated among non-ExPEC/UPEC isolates, whereas STc38, STc405, and STc648 predominated among ExPEC/UPEC isolates. Consistent with the virulence gene–based criteria for ExPEC and UPEC, all extraintestinal virulence genes studied were twice or more as prevalent among ExPEC/UPEC isolates as among non-ExPEC/UPEC isolates.

Clinical ESBL-PE Isolates From Medical Record Review

Our 10-year medical record review (5 years before and after travel) identified 4 ESBL-PE isolates in clinical samples collected from a presumed focus of infection during routine medical care. At the time of the present study, however, the corresponding 4 subjects all fell within the ESBL$^-$ group. For 3, the ESBL-PE–positive clinical sample (2, urine; 1, perianal abscess) had been collected before study participation. These patients had visited Northern Africa (2 patients) and South Asia (1 patient) within 3 months before the ESBL-PE infection. The fourth developed ESBL-PE bacteremia and peritonitis (related to appendiceal perforation) 4 years after study participation, and 2 months after visiting sub-Saharan Africa.

Table 2. Demographics, Destinations, and Symptoms in Relation to Pathotype Among 90 International Travelers

Host Characteristic	Subjects[a,b]	ESBL-producing *E. coli* Strains[c]	Strain Pathotype			
			Not DEC or ExPEC/UPEC[c]	DEC Only[c]	ExPEC/UPEC Only[c]	DEC and ExPEC/UPEC[c]
Total	90 (100)	96 (100)	47 (49)	10 (11)	36 (37)	3 (3)
Female gender	49 (54)	54 (56)	24 (51)	5 (50)	24 (67)	1 (33)
Age <50 y	59 (66)	64 (67)	29 (62)	7 (70)	2 (67)	24 (73)
Travel destination						
South Asia	28 (31)	29 (30)	12 (26)	8 (80)	9 (25)	0 (0)
Southeast Asia	33 (37)	37 (38)	18 (38)	1 (10)	16 (44)	2 (67)
Sub-Saharan Africa	23 (26)	24 (25)	14 (29)	0 (0)	10 (28)	0 (0)
East Asia	2 (2)	2 (2)	0 (0)	1 (10)	1 (3)	0 (0)
North Africa and Middle East	4 (4)	4 (4)	3 (6)	0 (0)	0 (0)	1 (33)
TD during travel[d]	75 (83)	81 (84)	36 (77)	10 (100)	33 (92)	2 (67)
UTI during travel	3 (3)	3 (3)	3 (6)	0 (0)	0 (0)	0 (0)
Antibiotic use during travel[e]	28 (31)	32 (34)	15 (33)	2 (20)	15 (42)	0 (0)

Data are presented as no. (%).

Abbreviations: DEC, diarrheagenic *Escherichia coli*; ESBL-PE, extended-spectrum β-lactamase producing Enterobacteriaceae; ExPEC, extraintestinal pathogenic *Escherichia coli*; TD, travelers' diarrhea; UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection.

[a]The 90 volunteers acquired a total of 99 ESBL-producing Enterobacteriaceae strains, of which 96 were *E. coli*, 1 was *Escherichia hermannii*, and 2 were *Klebsiella oxytoca*. Each of the carriers of the latter 3 strains also had an ESBL-producing *E. coli* strain. Six volunteers had 2 different ESBL-producing *E. coli* strains.

[b]Data are given per subject.

[c]Data are shown per strain.

[d]Typical of TD, many volunteers had concomitant stool pathogens other than the ESBL-DEC shown here.

[e]Information missing for 1 traveler who had 2 ESBL-PE strains.

Additionally, during the 5-year follow-up, routine stool screening cultures upon hospital admission detected ESBL-PE colonization in 3 subjects (2 patients, $ESBL^-$ group; 1 patient, $ESBL^+$ group). All 3 had recently revisited the tropics.

DISCUSSION

Despite growing concern about the surge of antimicrobial resistance among clinical isolates, many clinicians overlook the potential risks asymptomatic intestinal colonization by ESBL-PE

Table 3. Antibiotic Resistance Profile in Relation to Pathotype Among Extended-spectrum β-Lactamase–producing *Escherichia coli* Strains Acquired During International Travel[a]

Resistance Genotype[b] or Phenotype[c]	Total	Trait Prevalence by Pathotype Category				4-Group Comparison *P* Value
		Not DEC or ExPEC/UPEC	DEC Only	ExPEC/UPEC Only	DEC + ExPEC/UPEC	
Total	96 (100)	47 (49)	10 (11)	36 (37)	3 (3)	
Resistance genes						
TEM	48 (50)	25 (53)	3 (30)	18 (50)	2 (67)	.55
OXA	22 (23)	11 (23)	0 (0)	11 (31)	0 (0)	.17
SHV	4 (4)	2 (4)	1 (10)	1 (3)	0 (0)	.78
CTX-M group 1	67 (70)	30 (64)	8 (80)	28 (78)	1 (33)	.23
CTX-M group 9	26 (27)	13 (28)	1 (10)	10 (28)	2 (67)	.28
Resistant or intermediate to antibiotic						
Carbapenem	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Tobramycin[e]	49 (51)	23 (49)	2 (20)	24 (67)	0 (0)	.042[d]
Ciprofloxacin	64 (67)	33 (70)	3 (30)	28 (78)	0 (0)	.003[d]
Nitrofurantoin	2 (2)	2 (4)	0 (0)	0 (0)	0 (0)	.55
Cotrimoxazole	71 (74)	32 (68)	5 (50)	31 (86)	1 (33)	.054[d]

Data are presented as no. (%) unless otherwise indicated.

Abbreviations: DEC, diarrheagenic *Escherichia coli*; ExPEC, extraintestinal pathogenic *Escherichia coli*; NA, not applicable; TD, travelers' diarrhea; UPEC, uropathogenic *Escherichia coli*.

[a]Data are given per strain.

[b]No strains carried CTX-M group 2, 8, or 25; 1 "DEC only" strain carried DHA; 2 ESBL-PE strains with a negative pathotype analysis; and 1 each "UPEC only" and "ExPEC + UPEC" strains carried CIT.

[c]Isolates testing as resistant or intermediate were recorded as resistant.

[d]For DEC-only and DEC + ExPEC/UPEC strains combined vs ExPEC/UPEC-only strains: for tobramycin (2/13 vs 24/36), $P = .003$; for ciprofloxacin (3/13 vs 28/36), $P = .001$; and for cotrimoxazole (6/13 vs 31/36), $P = .008$ (Fisher exact test, 2-tailed).

[e]Tobramycin data missing for 1 isolate.

Table 4. Phylogroups, Sequence Type Complexes, and Virulence Genes of 96 Extended-spectrum β-Lactamase–producing *Escherichia coli* Strains Acquired During International Travel

Trait Category and Specific Trait	Total	Trait Prevalence by Pathotype Category				*P* Value
		Not DEC or ExPEC/UPEC	DEC Only	ExPEC/UPEC Only	DEC + ExPEC/UPEC	
Total	96 (100)	47 (49)	10 (11)	36 (37)	3 (3)	
Phylogroup[a]						
A	39 (41)	33 (70)	4 (40)	2 (6)	0 (0)	< .001
B1	12 (12)	9 (19)	3 (30)	0 (0)	0 (0)	.02
D	22 (23)	2 (4)	2 (20)	15 (42)	3 (100)	< .001
F	15 (15)	0 (0)	0 (0)	15 (42)	0 (0)	< .001
ST complex						
STc10	28 (29)	24 (51)	2 (20)	2 (6)	0 (0)	< .001
STc38	6 (6)	0 (0)	1 (10)	4 (11)	1 (33)	.031
STc405	7 (7)	0 (0)	0 (0)	7 (19)	0 (0)	.005
STc648	12 (12)	0 (0)	0 (0)	12 (33)	0 (0)	< .001
Virulence genes						
papAH/C	4 (4)	1 (2)	0 (0)	2 (6)	1 (33)	.06
afa/draBC	12 (12)	1 (2)	0 (0)	11 (31)	0 (0)	.001
yfcV	19 (20)	0 (0)	0 (0)	18 (50)	1 (33)	< .001
fyuA	57 (59)	20 (43)	4 (40)	30 (83)	3 (100)	.001
chuA	41 (43)	2 (4)	2 (20)	34 (94)	3 (100)	< .001
iutA	45 (47)	15 (32)	3 (30)	26 (72)	1 (33)	.003
kpsM II	32 (33)	4 (8)	3 (30)	22 (61)	3 (100)	< .001
vat	2 (2)	0 (0)	0 (0)	2 (6)	0 (0)	.31

Data are presented as no. (%) unless otherwise indicated.

Abbreviations: *afa/dra,* Dr-binding adhesins; *chuA,* heme uptake; DEC, diarrheagenic *Escherichia coli*; ExPEC, extraintestinal pathogenic *Escherichia coli*; *fyuA,* yersiniabactin system; *iutA,* aerobactin siderophore system; *kpsM* II, group 2 capsule; NA, not applicable; *papAH* and/or *papC,* P fimbriae structural subunit and assembly; *sfa/foc,* S and F1C fimbriae; STc, sequence type complex; TD, travelers' diarrhea; UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection; *vat,* vacuolating toxin; *yfcV,* adhesin.

[a]Phylogroups and ST complexes shown are those represented by ≥5 isolates each. Phylogroups represented by <5 strains each (number of strains): B2 (3), C (4), E (1). ST complexes represented by <5 isolates each: STc23, STc31, and STc206 (4 isolates each); STc131, STc155, and STc448 (3 isolates each); STc69, STc469, and STc490 (2 isolates each); and STc46, STc70, STc86, STc117, STc120, STc205, STc349, STc354, STc446, STc522, STc747, STc875, STc977, STc1140, STc1722, STc3716, and "novel" (1 isolate each).

may pose to travelers [31]. Our finding that a large proportion of imported ESBL-PE strains qualify molecularly as presumptive intestinal or extraintestinal pathogens suggests that more travel-acquired ESBL-PE may cause infections than has been recognized previously.

We found that 13 of 90 (14%) of the travel-acquired ESBL-PE strains were DEC, usually EAEC, a known cause of TD [18, 19, 32]. Most ESBL-producing EAEC strains originated in South Asia, which has the highest rates reported for both TD [17] and ESBL-PE acquisition [1], and here had the highest proportion of DEC among travel-acquired ESBL-PE strains (28% vs 7% for other regions combined).

Recent research on TD has documented stool pathogens among both symptomatic and asymptomatic travelers [18, 19], rendering causality ambiguous in individual cases. However, at the population level, in appropriately controlled studies, EAEC [18, 19, 32] and other intestinal pathogens [18, 19] have been more prevalent among symptomatic than asymptomatic travelers. Here, 12 of 13 (92%) volunteers with ESBL-producing DEC had TD, although (as is typical of TD) 10 of 13 had other potentially contributory stool pathogens (Supplementary Table 1). Thus, although the ESBL-producing DEC strain may not have accounted for the symptoms in all 12 TD cases, it probably contributed to some, especially the 3 in which ESBL-producing EAEC was the sole pathogen detected. Indeed, we have previously shown within the same cohort, in a 382-subject subgroup without antibiotic use, DEC strains to be significantly associated with TD (for EAEC: odds ratio [OR], 2.6 [95% confidence interval {CI}, 1.5–4.4]); for ETEC: OR, 5.9 [95% CI, 2.9–11.9]; and for EPEC: OR, 1.7 [95% CI, 1.0–2.7]) [19].

Others have also recently identified ESBL production by DEC strains [33]. Given the increasing prevalence of antimicrobial resistance among diarrheagenic pathogens [21, 22, 34, 35], this phenotype strikes us as especially worrying, since it further narrows treatment options if antimicrobial therapy is indicated.

Almost half (41%) of all travel-acquired ESBL-producing *E. coli* exhibited the ExPEC and/or UPEC pathotype, suggesting an increased potential for causing extraintestinal disease. However, among $ESBL^+$ group subjects, the only documented extraintestinal infections typical of Enterobacteriaceae were 3 cases of cystitis, all in subjects whose ESBL-producing strain was not ExPEC/UPEC. Allowing for the possibility that these subjects' non-ExPEC/UPEC ESBL-PE strains caused the cystitis episode, our data suggest a 3% (3/90) maximum incidence for extraintestinal infections due to travel-acquired ESBL-producing *E. coli*. Of note, this low rate appears

applicable only to healthy travelers, since in our recent study of ill subjects hospitalized abroad, up to 11% of travelers carrying a resistant strain (either ESBL- or carbapenemase-producing Enterobacteriaceae) had an extraintestinal infection recorded [36].

In previous reports, most carriers of travel-acquired ESBL-PE strains have remained asymptomatic [1]; symptomatic/clinical ESBL-PE infections have been considered rare [7, 14], with UTI as the most common clinical manifestation [15, 37]. Our data suggest a theoretical maximum rate of 17% for any symptomatic ESBL-PE infection during travel (TD: 12 [13.3%]; UTI: 3 [3.3%]) among our 90 travelers with travel-acquired ESBL-PE.

We recognize we may have missed some cases in which ESBL-producing DEC caused TD but were no longer detected upon the subjects' return to Finland, since travel-acquired ESBL-PE sometimes disappear during travel (A. Kantele, unpublished observation). This may be more likely for certain DEC types than others; for example, EAEC apparently persists longer than ETEC [18]. Likewise, because the posttravel stools were collected soon after return, we may have failed to capture some nonsevere cystitis and TD episodes, if symptoms developed only after stool sampling (ie, we may have detected the incipient pathogen during its latent/incubation phase, unaware that it later caused an infection). With respect to TD, since 12 of 13 subjects with ESBL-DEC had already reported TD during travel, data on (later-occurring) posttravel symptoms could theoretically have added at the most only the single subject who had not reported TD during travel.

Our theoretical maximum rate of 12 TD and 3 UTI cases caused by travel-acquired ESBL-PE among 90 colonized subjects contradicts the common view that UTI is the most frequent clinical consequence of such colonization [15]. Indeed, although this is evidently the case in developed countries [37], our results—a novel observation—suggest that for travelers visiting tropical regions, TD outnumbers UTI as a clinical correlate of travel-acquired ESBL-PE. This appears logical as TD pathogens directly reach their target—ie, the intestinal mucosa—while the pathogenesis of UTI requires that the colonizing strains access the urinary tract.

All 3 strains with overlapping intestinal and extraintestinal pathotypes qualified as EAEC, an established association [38, 39]. Of note, we have not encountered an overlap between ExPEC/UPEC and other DEC pathotypes than EAEC. Although the selective forces that favor such EAEC-ExPEC/UPEC hybrid strains remain undefined, EAEC-related adhesins may confer both uroepithelial and enterocyte adherence, and EAEC can cause UTI [32].

The observed phylogenetic segregation of colonizing DEC strains (groups A and B1; STc10) vs ExPEC/UPEC strains (groups D and F; STc38, STc405, STc648) accords with patterns identified among clinical isolates [40]. It supports the concept that these divergent pathotypes are mainly derived from distinct *E. coli* lineages, with the gut microbiota representing the (shared) primary reservoir.

Our 10-year medical record survey (spanning 5 pretravel and 5 posttravel years) was unlikely to have missed many severe posttravel infections treated at other institutions, since these medical records cover approximately 90% of all ESBL-PE isolates from clinical samples in the Uusimaa region (Martti Vaara, HUSLAB, personal communication). Furthermore, the 5-year posttravel survey window should suffice because of the transience of ESBL-PE colonization (median duration, 3 months), with a 1-year posttravel prevalence of only approximately 10% [10]. The laboratory records would, however, have missed mild diseases such as cystitis, which in most instances is treated empirically per international recommendations, without culture [25]. Accordingly, cystitis cases were reliably captured only during travel, by our prospective symptom surveillance.

Our medical record survey identified no more than 4 confirmed ESBL-PE infections unrelated to the index travel, all (paradoxically) among subjects in the ESBL$^-$ group. All 4 had traveled to a high-risk tropical area within 3 months before their clinical ESBL-PE infection, suggesting that they had contracted the causative strain during that journey. These data exemplify nicely the dynamic and paradoxical nature of the situation. Specifically: (1) each journey to an endemic area poses an appreciable risk of contracting ESBL-PE; (2) half of such strains are pathogenic *E. coli* (ExPEC/UPEC > DEC); (3) ESBL-PE colonization, although transient, confers a nonzero risk of clinical infection (TD more likely than extraintestinal infection); and (4) new travel involves new exposure and, thus, renewed risk of colonization and, possibly, infection.

CONCLUSIONS

In 13 of 90 (14%) returning Finnish travelers with travel-acquired ESBL-PE, the strains were DEC (mainly EAEC), with a high associated TD attack rate (92%). By contrast, in 40% the strains represent ExPEC and/or UPEC, yet with no associated clinical disease (extraintestinal infection attack rate, 0%). The only potential index travel–associated extraintestinal ESBL-PE infections were 3 cases of cystitis, involving subjects whose travel-acquired ESBL-producing *E. coli* strain was neither ExPEC/UPEC nor DEC. Thus, among travel-acquired ESBL-PE strains, the most frequent pathotype is ExPEC/UPEC, yet pathotype-consistent clinical manifestations are more common among hosts colonized by DEC than ExPEC/UPEC strains. The maximum total rate of ESBL-PE infections among colonized individuals was estimated at 17%; instead of UTI, the most commonly associated clinical manifestation of travel-acquired ESBL-PE was diarrhea.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the

posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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References

1. Woerther PL, Andremont A, Kantele A. Travel-acquired ESBL-producing Enterobacteriaceae: impact of colonization at individual and community level. J Travel Med **2017**; 24:29–34.
2. Tängdén T, Cars O, Melhus A, Löwdin E. Foreign travel is a major risk factor for colonization with *Escherichia coli* producing CTX-M-type extended-spectrum beta-lactamases: a prospective study with Swedish volunteers. Antimicrob Agents Chemother **2010**; 54:3564–8.
3. Tham J, Odenholt I, Walder M, Brolund A, Ahl J, Melander E. Extended-spectrum beta-lactamase-producing *Escherichia coli* in patients with travellers' diarrhoea. Scand J Infect Dis **2010**; 42:275–80.
4. Ostholm-Balkhed A, Tärnberg M, Nilsson M, Nilsson LE, Hanberger H, Hällgren A; Travel Study Group of Southeast Sweden. Travel-associated faecal colonization with ESBL-producing Enterobacteriaceae: incidence and risk factors. J Antimicrob Chemother **2013**; 68:2144–53.
5. Paltansing S, Vlot JA, Kraakman ME, et al. Extended-spectrum β-lactamase-producing Enterobacteriaceae among travelers from the Netherlands. Emerg Infect Dis **2013**; 19:1206–13.
6. Kuenzli E, Jaeger VK, Frei R, et al. High colonization rates of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in Swiss travellers to South Asia—a prospective observational multicentre cohort study looking at epidemiology, microbiology and risk factors. BMC Infect Dis **2014**; 14:528. doi:10.1186/1471-2334-14-528.
7. Kantele A, Lääveri T, Mero S, et al. Antimicrobials increase travelers' risk of colonization by extended-spectrum betalactamase-producing Enterobacteriaceae. Clin Infect Dis **2015**; 60:837–46.
8. Ruppé E, Armand-Lefèvre L, Estellat C, et al. High rate of acquisition but short duration of carriage of multidrug-resistant Enterobacteriaceae after travel to the tropics. Clin Infect Dis **2015**; 61:593–600.
9. Reuland EA, Sonder GJ, Stolte I, et al. Travel to Asia and traveller's diarrhoea with antibiotic treatment are independent risk factors for acquiring ciprofloxacin-resistant and extended spectrum beta-lactamase-producing Enterobacteriaceae—a prospective cohort study. Clin Microbiol Infect **2016**; 22:731.e1–7.
10. Arcilla MS, van Hattem JM, Haverkate MR, et al. Import and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): a prospective, multicentre cohort study. Lancet Infect Dis **2017**; 17:78–85.
11. Woerther PL, Burdet C, Chachaty E, Andremont A. Trends in human fecal carriage of extended-spectrum β-lactamases in the community: toward the globalization of CTX-M. Clin Microbiol Rev **2013**; 26:744–58.
12. Schwaber MJ, Navon-Venezia S, Kaye KS, Ben-Ami R, Schwartz D, Carmeli Y. Clinical and economic impact of bacteremia with extended- spectrum-beta-lactamase-producing Enterobacteriaceae. Antimicrob Agents Chemother **2006**; 50:1257–62.
13. Epelboin L, Robert J, Tsyrina-Kouyoumdjian E, Laouira S, Meyssonnier V, Caumes E; MDR-GNB Travel Working Group. High rate of multidrug-resistant gram-negative bacilli carriage and infection in hospitalized returning travelers: a cross-sectional cohort study. J Travel Med **2015**; 22:292–9.
14. Vading M, Kabir MH, Kalin M, et al. Frequent acquisition of low-virulence strains of ESBL-producing *Escherichia coli* in travellers. J Antimicrob Chemother **2016**; 71:3548–55.
15. Søraas A, Sundsfjord A, Sandven I, Brunborg C, Jenum PA. Risk factors for community-acquired urinary tract infections caused by ESBL-producing Enterobacteriaceae—a case-control study in a low prevalence country. PLoS One **2013**; 8:e69581.
16. Osthoff M, McGuinness SL, Wagen AZ, Eisen DP. Urinary tract infections due to extended-spectrum beta-lactamase-producing gram-negative bacteria: identification of risk factors and outcome predictors in an Australian tertiary referral hospital. Int J Infect Dis **2015**; 34:79–83.
17. Steffen R, Hill DR, DuPont HL. Traveler's diarrhea: a clinical review. JAMA **2015**; 313:71–80.
18. Lääveri T, Antikainen J, Pakkanen SH, Kirveskari J, Kantele A. Prospective study of pathogens in asymptomatic travellers and those with diarrhoea: aetiological agents revisited. Clin Microbiol Infect **2016**; 22:535–41.
19. Lertsethtakarn P, Silapong S, Sakpaisal P, et al. Travelers' diarrhea in Thailand: a quantitative analysis using TaqMan array card. Clin Infect Dis **2018**; 67: 120–7.
20. Kantele A, Mero S, Kirveskari J, Lääveri T. Fluoroquinolone antibiotic users select fluoroquinolone-resistant ESBL-producing Enterobacteriaceae (ESBL-PE)—data of a prospective traveller study. Travel Med Infect Dis **2017**; 16:23–30.
21. Mendez Arancibia E, Pitart C, Ruiz J, Marco F, Gascón J, Vila J. Evolution of antimicrobial resistance in enteroaggregative *Escherichia coli* and enterotoxigenic *Escherichia coli* causing traveller's diarrhoea. J Antimicrob Chemother **2009**; 64:343–7.
22. Ouyang-Latimer J, Jafri S, VanTassel A, et al. In vitro antimicrobial susceptibility of bacterial enteropathogens isolated from international travelers to Mexico, Guatemala, and India from 2006 to 2008. Antimicrob Agents Chemother **2011**; 55: 874–8.
23. Margulieux KR, Srijan A, Ruekit S, et al. Extended-spectrum beta-lactamase prevalence and virulence factor characterization of enterotoxigenic *Escherichia coli* responsible for acute diarrhea in Nepal from 2001 to 2016. Antimicrob Resist Infect Control **2018**; 7:87. doi:10.1186/s13756-018-0377-2.
24. World Health Organization. Health topics: diarrhoea. Available at: http://www.who.int/topics/diarrhoea/en/. Accessed 28 October 2018.
25. Warren JW, Abrutyn E, Hebel JR, Johnson JR, Schaeffer AJ, Stamm WE. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Infectious Diseases Society of America (IDSA). Clin Infect Dis **1999**; 29:745–58.
26. Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. J Antimicrob Chemother **2010**; 65:490–5.
27. Antikainen J, Kantele A, Pakkanen SH, et al. A quantitative polymerase chain reaction assay for rapid detection of 9 pathogens directly from stools of travelers with diarrhea. Clin Gastroenterol Hepatol **2013**; 11:1300–7.e3.
28. Johnson JR, Johnston BD, Porter S, Thuras P, Aziz M, Price LB. Accessory traits and phylogenetic background predict *Escherichia coli* extraintestinal virulence better than does ecological source. J Infect Dis **2019**; 219:121–32.
29. Clermont O, Christenson JK, Denamur E, Gordon DM. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep **2013**; 5:58–65.
30. Johnson JR, Porter S, Thuras P, Castanheira M. Epidemic emergence in the United States of *Escherichia coli* sequence type 131-H30 (ST131-H30), 2000 to 2009. Antimicrob Agents Chemother **2017**; 61. doi:10.1128/AAC.00732,17.
31. DuPont HL, Steffen R. Use of antimicrobial agents for treatment and prevention of travellers' diarrhoea in the face of enhanced risk of transient fecal carriage of multi-drug resistant Enterobacteriaceae: setting the stage for consensus recommendations. J Travel Med **2017**; 24:57–62.
32. Hebbelstrup Jensen B, Olsen KE, Struve C, Krogfelt KA, Petersen AM. Epidemiology and clinical manifestations of enteroaggregative *Escherichia coli*. Clin Microbiol Rev **2014**; 27:614–30.

33. Guiral E, Mendez-Arancibia E, Soto SM, et al. CTX-M-15-producing enteroaggregative *Escherichia coli* as cause of travelers' diarrhea. Emerg Infect Dis **2011**; 17:1950–3.
34. Gunell M, Aulu L, Jalava J, et al. Cefotaxime-resistant *Salmonella enterica* in travelers returning from Thailand to Finland. Emerg Infect Dis **2014**; 20:1214–7.
35. Post A, Martiny D, van Waterschoot N, et al. Antibiotic susceptibility profiles among *Campylobacter* isolates obtained from international travelers between 2007 and 2014. Eur J Clin Microbiol Infect Dis **2017**; 36:2101–7.
36. Khawaja T, Kirveskari J, Johansson S, et al. Patients hospitalized abroad as importers of multiresistant bacteria—a cross-sectional study. Clin Microbiol Infect **2017**; 23:673.e1–8.
37. Rodríguez-Baño J, Pascual A. Clinical significance of extended-spectrum beta-lactamases. Expert Rev Anti Infect Ther **2008**; 6:671–83.
38. Wallace-Gadsden F, Johnson JR, Wain J, Okeke IN. Enteroaggregative *Escherichia coli* related to uropathogenic clonal group A. Emerg Infect Dis **2007**; 13: 757–60.
39. Olesen B, Scheutz F, Andersen RL, et al. Enteroaggregative *Escherichia coli* O78:H10, the cause of an outbreak of urinary tract infection. J Clin Microbiol **2012**; 50:3703–11.
40. Johnson JR, Russo TA. Molecular epidemiology of extraintestinal pathogenic *Escherichia coli*. EcoSal Plus **2018**; 8. doi:10.1128/ecosalplus.ESP,0004-2017.